Effect of erythromycin treatment delay on therapeutic outcome of experimental acute otitis media caused by Streptococcus pneumoniae

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Objective: To evaluate the effect of delayed administration of erythromycin in the course of acute otitis media caused by an erythromycin-susceptible Streptococcus pneumoniae strain in the gerbil model.

Methods: The bacterium was inoculated by transbullar challenge in the middle ear (ME) and antibiotic treatment at different doses was administered at various times thereafter.

Results: When 2.5 mg/kg of erythromycin was administered as a single dose 2, 5, 18 or 21 h post-inoculation (pi) the bacterial eradication rate was 55, 40, 0 and 0%, respectively. A higher dose (5 mg/kg) administered also as a single dose 2, 5, 18 and 21 h pi achieved bacterial eradication rates of 62.5, 43.8, 0 and 0%, respectively. Using a very high dose (50 mg/kg) repeated three times at 3 h intervals (total dose 150 mg/kg) and starting the treatment 21 h pi only achieved bacterial eradication in 25% of cases. The concentration of erythromycin achieved in the ME 90 min after administration of 5 mg/kg 5 or 21 h pi was very similar (0.74 and 0.79 mg/L) but the ME half-life was longer (98.2 min) with the early administration as compared with the delayed form (47.5 min), which could partially explain the different results. Further experiments showed that the failures observed with the delayed administration were not related to the time elapsed from antibiotic administration to ME sampling or selection of antibiotic-resistant mutants.

Conclusion: Bacteriological and clinical efficacies were significantly diminished if antibiotic administration was delayed.

Keywords: otitis, pneumococcal, therapy delay, gerbils

Introduction

Acute otitis media (AOM) is one of the most frequent illnesses of childhood, the predominant bacterium involved being Streptococcus pneumoniae.1 As many cases of AOM will resolve spontaneously, it has been suggested that treatment with antimicrobials should be delayed awaiting a possible clinical improvement.2,3 However, the effect of delayed antibiotic treatment has not been studied. We have previously shown that the efficacy of amoxicillin in the treatment of experimental AOM caused by S. pneumoniae decreased with delayed antibiotic treatment.4

The aim of this study was to evaluate the effect of delayed administration of erythromycin in the course of an experimental AOM caused by an erythromycin-susceptible S. pneumoniae strain.

Materials and methods

Bacteria

A strain of S. pneumoniae (serotype 23F) (erythromycin MIC 0.12 mg/L) was used.

Antibiotic

Erythromycin (Sigma Chemicals Co., St Louis, MO, USA) and erythromycin lactobionate (Pantomicina; Abbott Laboratories, S.A., Madrid, Spain) were used for in vitro and in vivo studies.

In vitro studies

Erythromycin MICs were determined in 32 isolates collected from treated animals without complete bacterial eradication and in four from controls.
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**Animals**

Mongolian gerbils were purchased from the Centre d’Élevage R. Janvier (Le Genest, St-Isle, France) and managed as previously described. The study was performed following current regulations regarding the use of laboratory animals in the European Community and was approved by our Ethics Committee.

**Experimental otitis**

Animals were inoculated bilaterally into the middle ear (ME) bulla with ~10⁶ cfu of S. pneumoniae in 20 μL following published methods.¹⁵

**Treatment regimens and efficacy studies**

Erythromycin was given subcutaneously as a single dose (2.5 and 5 mg/kg) administered at 2, 5, 18 and 21 h post-inoculation (pi). Experiments using three infusions of 5 and 50 mg/kg (total dose of 15 and 150 mg/kg, respectively) at different times (2, 5 and 8, or 21, 24 and 27 h pi) were also carried out.

Animals in the control groups received pyrogen-free sterile distilled water. Groups of 8–10 animals per treatment and control groups were included. Efficacy was evaluated at 48 h pi for single and repeated doses. Other groups of animals were evaluated at 29 h pi after repeated doses administered at 2, 5 and 8 h pi. Treated and control animals were evaluated for otorrhoea, weight and otoscopic appearance. Otopsychic features were evaluated as previously defined¹⁵ and ME washings were obtained from both ears at 29 and 48 h pi and plated for colony counting.

**Pharmacokinetic studies**

Serum levels of erythromycin were determined in healthy animals after a single subcutaneous injection of 5 mg/kg of the antibiotic. Groups of six animals per dose had blood samples obtained at 15, 30, 60 and 120 min after drug administration. Antibiotic concentrations in ME fluid (MEF) were determined in groups of 10 animals bilaterally inoculated with the organism. A single 5 mg/kg dose of erythromycin was subcutaneously administered 5 or 21 h pi and MEF samples were obtained 60, 90 and 120 min thereafter. Aliquots of MEF samples were pooled for determination of antibiotic levels. Antibiotic concentrations were determined by microbiological assay. Assay variability for individual samples was <10%.

**Results**

**In vitro studies**

The erythromycin MIC values for all isolates from control and treated animals remained at 0.12 mg/L.

**Experimental otitis and therapeutic efficacy**

After inoculation of 6.22 ± 0.36 log₁₀ cfu (mean ± SD of all experiments) bilateral AOM and otorrhoea was observed in all untreated animals that showed lethargy and significant weight loss. Table 1 presents the bacteriological and clinical efficacy of erythromycin administered as a single dose at different intervals. A significant reduction in otorrhoea only occurred in those animals treated with any dose 2 h after bacterial challenge as compared with untreated controls. A significant reduction in the AOM was observed only in those animals treated with 5 mg/kg of erythromycin administered 2 h pi as compared with any other groups. Animals treated with any dose between 2 and 5 h pi showed significant reduction in the number of culture-positive ears as compared with any other group within the same dose and untreated control. A significant reduction in the number of organisms recovered from the ME, as compared with untreated controls, was observed after administration of 2.5 mg/kg 2 h pi, and

**Table 1. Bacteriological and clinical efficacies⁶ of erythromycin administered in two doses at different intervals after bacterial inoculation**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Challenge to treatment (h)</th>
<th>Otorrhoea (%)</th>
<th>Percentage of</th>
<th>Culture-positive ME samples (%)</th>
<th>Mean bacterial count (log_{10} no. of cfu per 20 μL) ± SD</th>
<th>Mean body weight loss (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>NA b</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
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<td>2.5</td>
<td>2</td>
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<td>75</td>
<td>15</td>
<td>10</td>
<td>45 d</td>
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<td></td>
<td>5</td>
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<td>10</td>
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<tr>
<td>5</td>
<td>2</td>
<td>53.3 c</td>
<td>18.8 d</td>
<td>25</td>
<td>56.2</td>
<td>37.5 d</td>
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<tr>
<td></td>
<td>5</td>
<td>87.5</td>
<td>75</td>
<td>6.2</td>
<td>18.8</td>
<td>56.2 d</td>
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<td></td>
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<td>93.8</td>
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</tr>
</tbody>
</table>

AOM, acute otitis media; IOM, intermediate otitis media; OME, otitis media with effusion.

bNA, not applicable.

¹⁵Significant difference (P < 0.05) from value for untreated control.

⁶Significant difference (P < 0.01) from values for any other group within the same dose and untreated control.
Delayed erythromycin treatment in pneumococcal otitis media

Table 2. Bacteriological and clinical efficacies of erythromycin administered in doses of 5 and 50 mg/kg in three shots (total dose of 15 and 150 mg/kg, respectively) at early (2, 5 and 8 h) or delayed (21, 24 and 27 h) starting times after bacterial inoculation

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Challenge to treatment (h)</th>
<th>Ototithrea (%)</th>
<th>Percentage of</th>
<th>Culture-positive ME samples (%)</th>
<th>Mean bacterial count (log10 no. of cfu per 20 μL) ± SD</th>
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<tbody>
<tr>
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<td>NA b</td>
<td>100</td>
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<td>5</td>
<td>2</td>
<td>25 c</td>
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<td>87.5</td>
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<td>50</td>
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<td>87.5</td>
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</tbody>
</table>

AOM, acute otitis media; IOM, intermediate otitis media; OME, otitis media with effusion.

Pharmacokinetic and pharmacodynamic data

The serum concentration of erythromycin 15 min after administration of 5 mg/kg was 1.64 ± 0.21 mg/L with a half-life of 31 min and an AUC of 98.6 mg·min/L. This dose administered 5 or 21 h pi produced, 90 min thereafter, a MEF concentration of 0.74 or 0.79 mg/L. The half-lives and AUC/MIC ratios were 98.2 or 47.5 min, and 1236 or 732, which correlated with bacterial eradication of 43.8% and 0%, respectively.

Discussion

Most experiments were carried out using 2.5 or 5 mg/kg of erythromycin because such doses achieve serum and ME concentrations in gerbils similar to those obtained in children receiving the standard dose for the treatment of AOM.6–9 The best therapeutic regimen after once-only administration of the antibiotic was related to both time from challenge to treatment and erythromycin dose. When the antibiotic was administered 18 or 21 h pi neither a dose of 2.5 nor 5 mg/kg improved bacteriological and clinical results as compared with untreated controls.

The poorer efficacy observed with delayed administration of the antibiotic was not due to selection of subpopulations with higher MIC values. Furthermore, such results cannot be explained by the bacterial burden present at time of treatment initiation because the antibiotic efficacy was greater at 5 h than at 21 h, the bacterial burden being lower at later times in untreated controls.4 The delayed administration of three doses of either 5 or 50 mg/kg improves efficacy only slightly. The most significant pharmacokinetic/pharmacodynamic differences were the lower half-life and AUC values obtained when the antibiotic was delayed compared with early administration. Such differences could be due to the presence of otorrhoea at the time of delayed administration.

The better pharmacodynamic properties when 5 mg/kg of the antibiotic was administered early as a single dose compared with delayed administration could be responsible, in part, for the higher efficacy (bacterial eradication of 43.8% versus 0%). However when a higher dose was administered (5 mg/kg in three doses; total 15 mg/kg) in delayed form the eradication rate was not significantly improved (6.2%). Even administration of a very high dose (150 mg/kg) in three doses in delayed form increased the efficacy only modestly (25% eradication).

The clinical relevance of our results is unclear. The use of antibiotics in AOM is controversial but our results suggest that even if they are given in some cases, they should be administered as early as possible, as proposed by Hendley.10

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